HISTOPHYSIOLOGICAL STUDIES ON THE EFFECT OF ENVIRONMENTAL CONDITIONS ON TERRESTRIAL ISOPODES

by

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In earlier studies (Vitéz 1970) it was found thet of the activity of the neurosecretory cells in the cerebral ganglion of isopode species varied with the season. An attempt was made to answer the question of how environmental factors affect the activity of the neurosecretory cells of the brain. To this end we examined the influence of temperature and illumination under experimental conditions.

Materials and Methods

In the experiments sexually mature specimens of some terrestrial isopodes *(Porcellio dilatatus* Brandt, *Porcellio laevis* Latreille, *Protracheoniscus asiaticus* Uljanin) were used. The animals were fixed in Bouin or Susa solution. After applying Péterfi's double embedding method, we examined the methyl-benzoate-paraffinic, $5-6\ \mu$ thick sections by Gömöri's and Gabe's paraldehyde-fuchsin stain, and by Gömöri's chromehaematoxylin-phloxin stain, as well as by Sterba's pseudoisocyanine reaction.

Results

1. Effect of temperature

Our experiments were performed in a period between the end of October and middle of January. It is in this season that the terrestrial isopodes change over to the winter quiescent stage.

The animals were devided into three groups: those

a) kept at high temperature (32 °C)

b) kept at low temperature (4 °C)

c) kept at room temperature (23 °C)

a) *Effects of high temperature*

In the neurosecretory cells of the cerebral ganglia of the animals kept at ghigh temperature a stimulated secretion can be observed as compared to the control animals (Fig. 1). The cytoplasm of the "β" cells is full of small and large rough secretion-drops. The granules in the "A" cells are fairly rough, vacuoles at the edge of the cells are frequent. Beside the G ö m ö r i-positive granules also a secretion appears in the "B" cells, which fusion in the form of big phloxinophilic drops.

The "γ" cells of the lobus opticus stain very deep with paraldehyde-fuchsin. In these cells the secretory granules fusion with each other forming an uninterrupted egde of plasm around the nucleus.

In the tractus leading to the repository organ both acidophilic and basophilic secretion can be seen (Fig. 2). At the base of the sinus-gland there are some acidophilic drops while in the gland proper a great quantity of G ö m ö r i-positive material accumulates.

b) *The effect of low temperature*

As compared to the control the neurosecretory cells of the treated animals are less active. Although there is no reduction in volume of the cytoplasm of the "β" and "A" cells, the secretion is gradually evacuated and they are not filled again. Naturally, in a few cells some secretory granules remain. The cytoplasm of the "B" and "γ" cells contracts, retains a small quantity of secretion or evacuates completely (Fig. 3).

Fig. 1. "B" and "β" cells in the cerebral ganglion of animals kept at high temperature for 6 days. Susa. Paraldehyde-fuchsin.

In the tractus, however, there is some secretion, and the repositori organ does not evacuate either. It accumulates some well staining G ö m ö r i-positive material; the acidophilic granules present are quite few in number. In the brain of the winter animals a similar histological structure can be observed.

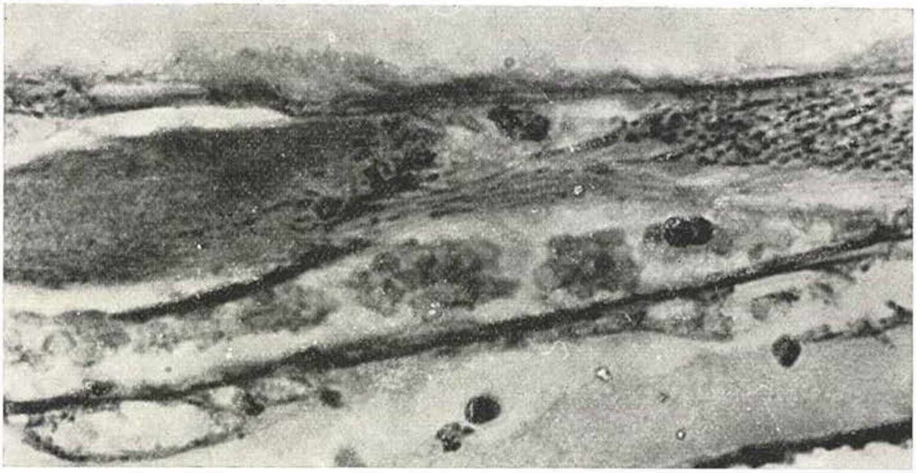

Fig. 2. Lobus opticus of the same animal with paraldehyde-fuchsin-positive colloid.

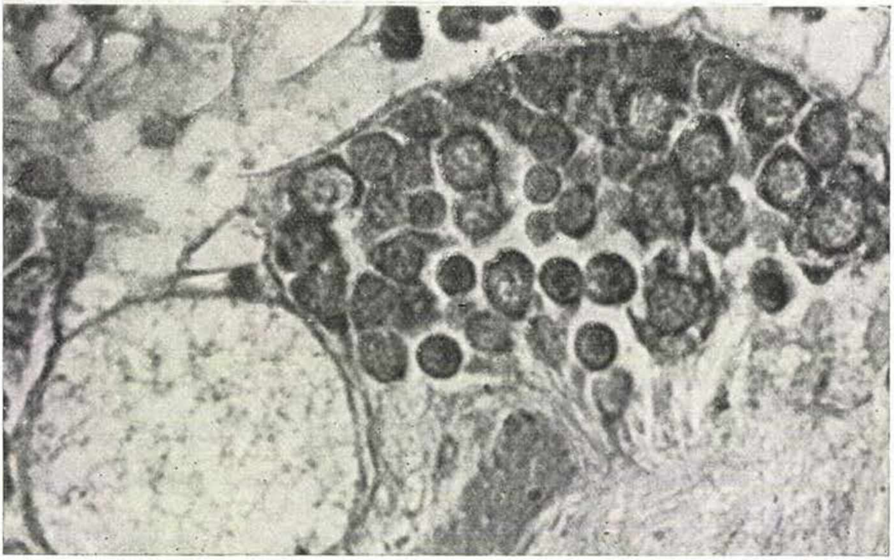

Fig. 3. "B" cells from the ganglion of an animal kept at low temperature for 10 days. Bouin. Paraldehyde-fuchsin.

2. *Effect of light*

The experiments were carried out in August and September. The experimental animals (50 individuals) were devided into several groups. Each group was placed in glass vessels fitted on the inside with wet filter-paper, and illuminated from a distance of 25 cm, for 4 – 100 hours

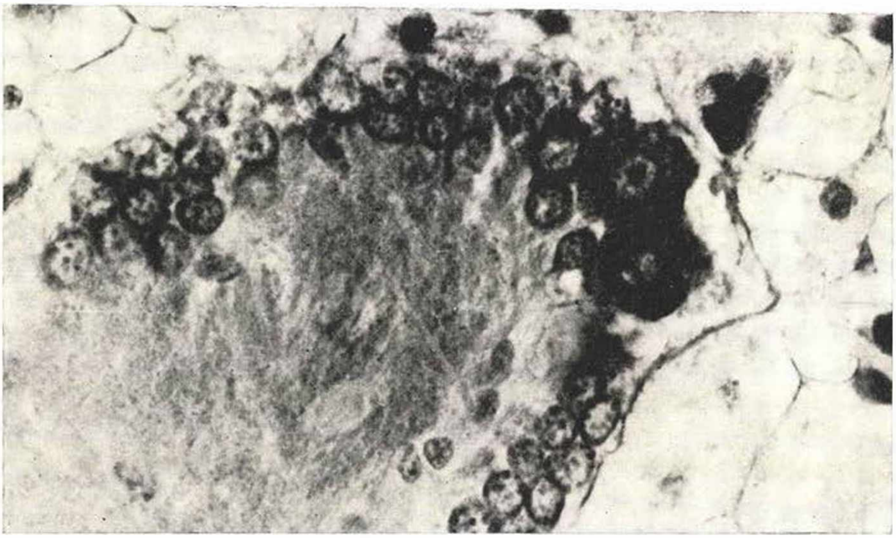

Fig. 4. "B" and "β" cells full of secretion (6 hours illumination.) Bouin. Paraldehyde-fuchsin.

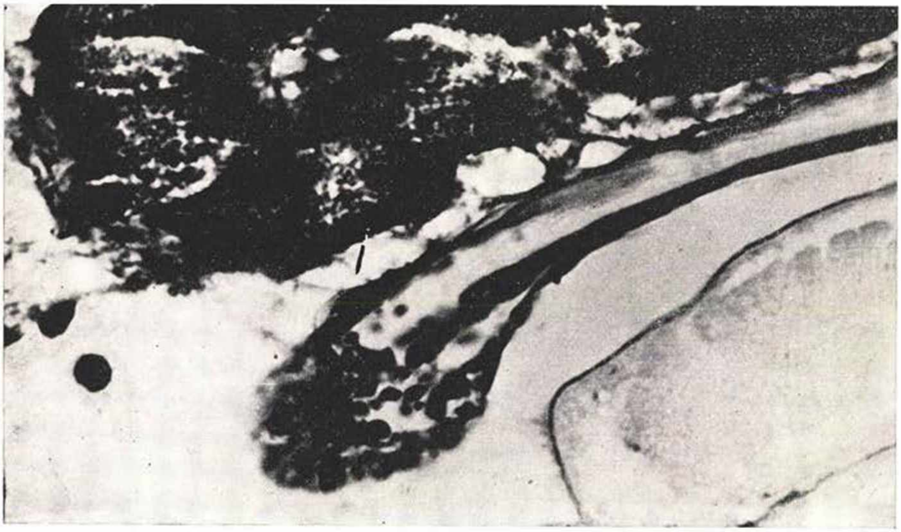

Fig. 5. The sinus-gland of the same animal. Paraldehyde-fuchsin.

by a 100W bulb (4 – 6 hours, 19 – 24 hours, 3 and 4 days). To eliminate heat effects, a vessel filled with ice water was interposed.

Immediately after termination of the illumination, the majority of the experimental animals were fixed by the method mentioned above, some of them were kept in darkness.

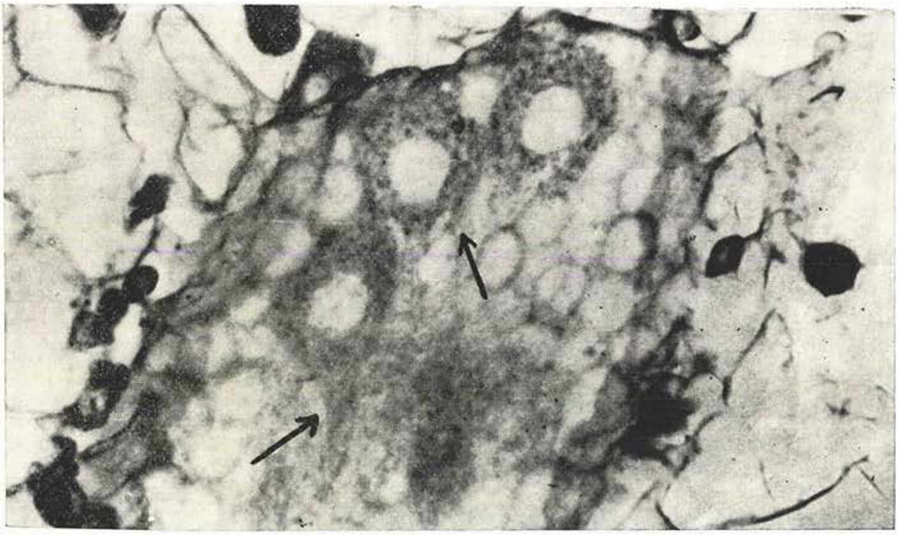

Fig. 6. Secretory granules in the cytoplasm and axon of "β" cells (20 hours illumination). Bouin. Paraldehyde-fuchsin.

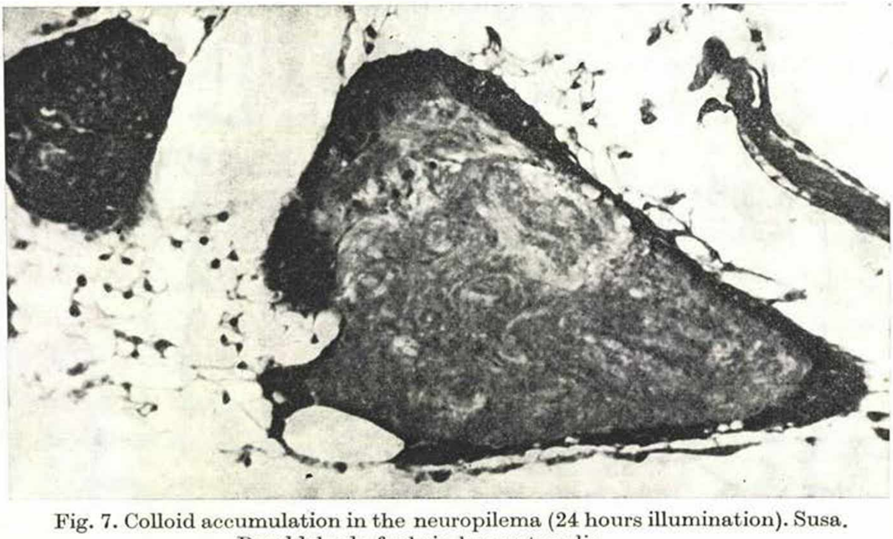

Fig. 7. Colloid accumulation in the neuropilema (24 hours illumination). Susa. Paraldehyde-fuchsin-haematoxylin.

Results of the experiment

Group 1 illuminated for 4 – 6 hours

The amount of the secretion present in the neurosecretory cells of the cerebral ganglion is the same as that in August (Fig. 4). In the cytoplasm of the "β" and "A" cells larger, in that of the "B" cells evenly distributed finer granular secretion can be found. The "γ" cells of the lobus opticus also stain deep in paraldehyde-fuchsin. In the lobus opticus and in the sinus gland a great amount of secretion appears (Fig. 5).

Group 2 illuminated for 19 – 24 hours

The activity of the neurosecretory cells is more intense. Every cell is full of secretion-drops. The secretion penetrates even into the thick, big axon of the "β" cells (Fig. 6). A large amount of basophilic and acidophilic stained colloidal substance accumulates also in the neuropilema under the ganglia (Fig. 7). In the lobus opticus the path of the axon of same cells can be fairly well followed even up to the lamina ganglionaris (Fig. 8). There are no such "row-of-pearl" – like formation in the control animals. Besides a number of basophilic drops, there are also a large number of acidophil granula, this time covering the whole area fo the "gland".

Group 3 illuminated for several days

Illumination for several days first brings about the hyperfunction of the neurosecretory cells, then it results in their total exhaustion. The cytoplasm of the cell evacuates, the neurosecretory granules are easy to follow in the processes of the cells. The maximum quantity of secre-

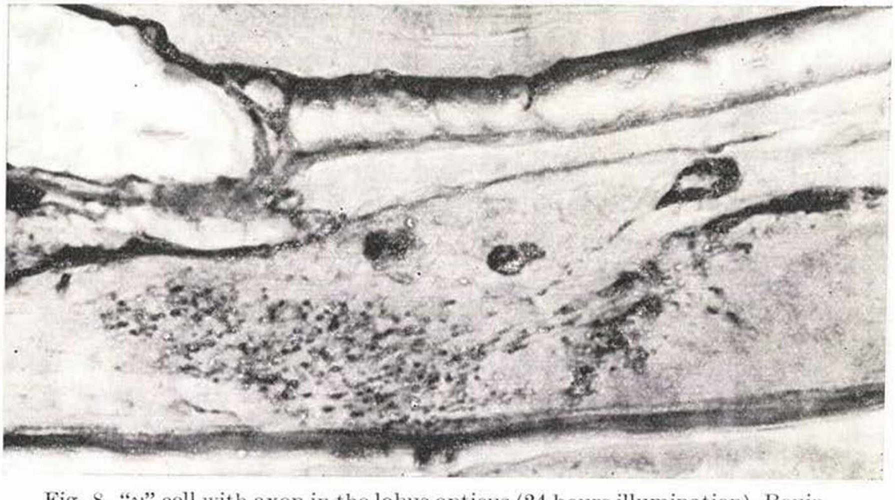

Fig. 8. "γ" cell with axon in the lobus opticus (24 hours illumination). Bouin. Paraldehyde-fuchsin.

tion accumulats in the ganglia, under the neuropilema in the lobus opticus and in the repository organ (Fig. 9). The sinus-gland is considerably enlarged, in certain cases it becomes berry-like (Fig. 10).

The structure of the cytoplasm and the nucleus in the neurosecretory cells of the cerebral ganglion of animals placed in darkness after continued illumination is normal. The cells regenerate, the changes are thus transitory.

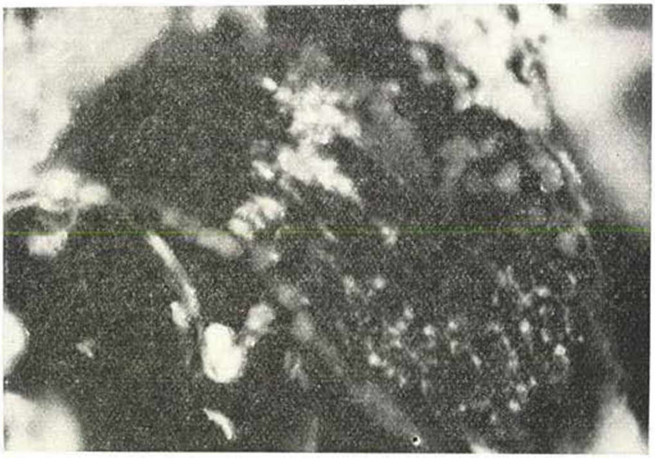

Fig. 9. Accumulation and transport of secretion in the lobus opticus (24 hours illumination). Bouin. Pseudoisocyanin reaction.

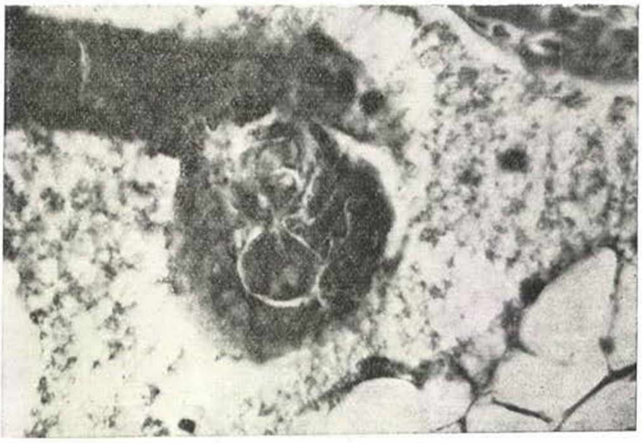

Fig. 10. Berry-like, enlarged simus gland (72 hours illumination). Bouin. Paraldehyde-fuchsin.

Discussion

Under experimental circumstances we studied the effect of light and temperature to throw some light on the role of environmental factors in the rhythm of the neurosecretory cells of the nervous system.

According to the literature, of the environmental conditions it is the duration and intensity of illumination, as well as the temperature and air humidity, that influence both the moulting and the reproductive process. Under experimental conditions it is possible with isopodes to evoke artificial reproduction even in winter (Wieser 1963, Beyer 1957/58). According to Passano (1960), light effects the activity of the ovary in a different way than it does sloughing.

In Wieser's opinion (1963), there is a "light-meter" in *Porcellio* reacting to the duration of illumination, while a system sensitive to temperature completes the activity of the former. In the opinion of several investigators (Henke 1933, Bayer 1957/58, Wieser 1963) it is on the first place temperature, that reproduction depens on.

Assimilar relation was found between the process of sloughing and the temperature and the illumination (Passano 1960). Low temperature inhibits, high temperature stimulates the sloughing activity of crabs. Also the time interval between the individual sloughings can shorten under the effect of high temperature. The sloughing of crabs is also influenced by the intensity, and duration of illumination. Daily light-periods which are longer than normal will stimulate the sloughing-activity in winter.

Beyer (1965) examined how light affets the development of terrestrial isopodes. He observed that in continous darkness their development is slower, in changing light, however, they grow more quickly, gain in weight, and also multiply.

In studies on the light tolerance of terrestrial isopodes, several investigators (Henke 1933, Bauers 1953) classified the species from this point of view, proving that *Oniscus* e.g. completely avoids light, *Porcellios* can adopt, while *Armadillidium* tolerates light fairly well. According to Bauers (1953) air temperature and humidity influence light tolerance.

The above experiments are not supplemented by histological examinations. We have, however, some literary references referring to what changes the various temperature and heat effects bring about in the neurosecretion cells. It was observed on fresh-water molluscs *(Anodonta cygnea)* (Polgár – Baranyi 1969), that raising the temperature 6 – 8 °C higher than the natural setting, promotes the accumulation and storing of neurosecretion and accelerates its evacuation. Low temperature accelerats the discharge of accumulated secretion too, but it also inhibits its reformation. Konok (1962) also found a relation between illumination and the histomorphological appeareance of the neuroendocrine-system in crabs *(Astacus leptodactylus)*. Among others, he noticed that the number of granulocytes increased in the haemolymph. From this he inferred that they play an important role in the enhanced transport of the chromatophorotrop hormone.

J. de Wilde (1965) in a study on the photoperiodic control of the endocrine system of insects was established that the neurosecretory cells of the brain of Leptinotarsa are activated by a long day. A short day does not only leave them inactive, but probably results in the inhibition of the activity of the coropra allata, shortening the praediapause.

In our experiments we actually attempted to imitate whithin certain limits the individual seasons. As the parts of the day and the seasons are characterised by a certain amount of illumination and a definite temperature, similarly in our experiment high temperature and prolonged illumination corresponds to summer, low temperature and darkness to autumn and winter months.

A comparison of our present results with those of our studies on seasonal rhythm shows that the histological structure of the brain of the animals kept at high temperature and illumination corresponds to that of summer animals, while it corresponds to winter animals in individuals kept at low temperature and in darkness. Prolonged illumination and very high temperature are no more similar to physiological conditions. The neurosecretory cells, however, are capable of adapting themselves to the environmental factors; though the cells get exhausted by overstrain, they regain their original structure and activity after these effects cease, and if these effects have not resulted in a permanent damage.

This adaptability of the neurosecretional system in the Invertebrata ensures the adaptation to changed circumstances. This is made safer by the combination of the two reactive systems (light and temperature).

Summary

Histophysiological studies have shown that there is a relationship between environmental factors, such as light and temperature on the one hand, and the activity of the neurosecretory cells of the brain, the accumulation and transport of the neurosecretion on the other.

a) In the brain of the animals kept at high temperature, in the cells as well as in the repository organ, a larger amount of neurosecretion is present, than in those kept at low temperature. These observations suggest that high temperature stimulates, low temperature inhibits the secretion process.

b) Illumination for a few hours stimulates the activity of the neurosecretory cells; illumination for several days leads to hyperfunction, later to complete exhaustion. These changes are of a transitory nature, for the cells regenerate after the illumination is over. It is suggested that in addition to the "endogenous rhythm" which envolved in the course of phylogenesis, the seasonal rhythm of the neurosecretion also greatly depends on environmental effects, such as light and temperature.

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